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The Effects of Hypothermia and Hyperthermia on
Energy Metabolism and Blood Flow in the Rat
Brain

Lund 1975

THE EFFECTS OF HYPOTHERMIA AND HYPERTHERMIA
ON ENERGY METABOLISM AND BLOOD FLOW IN THE RAT BRAIN

An experimental study

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RAT BRAIN

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THE PRESENT THESIS IS BASED ON THE FOLLOWING PAPERS:

- I. M. Hägerdal, J. Harp, L. Nilsson and B.K. Siesjö:
The effect of induced hypothermia upon oxygen consumption in the rat brain. Journal of Neurochemistry, 1975, 24, 311-316.
- II. M. Hägerdal, J. Harp and B.K. Siesjö:
Effect of hypothermia upon organic phosphates, glycolytic metabolites, citric acid cycle intermediates and associated amino acids in rat cerebral cortex. Journal of Neurochemistry, 1975, 24, 743-748.
- III. M. Hägerdal, J. Harp and B.K. Siesjö:
Influence of changes in arterial PCO_2 on cerebral blood flow and cerebral energy state during hypothermia in the rat. Acta anaesth. scand. 1975, suppl. 57, 25-33.
- IV. C. Carlsson, M. Hägerdal and B.K. Siesjö:
The effect of hyperthermia upon oxygen consumption and upon organic phosphates, glycolytic metabolites, citric acid cycle intermediates and associated amino acids in rat cerebral cortex. Journal of Neurochemistry, 1976. Accepted for publication.

In the text the papers are referred to by their Roman numerals.



INTRODUCTION

Hypoxia^x and ischemia^x are not uncommon complications after accidents, haemorrhage, or during operations. As the nerve cells are especially sensitive to lack of oxygen, it sometimes happens that serious brain damage remains, although the primary hypoxia or ischemia has been effectively treated. It is a common clinical observation that altered body temperature can influence the outcome in cerebral hypoxia and ischemia. Increased body temperature, hyperthermia, can be unfavourable, probably because as the metabolism increases the demand for both oxygen and substrate increases. On the other hand, it is also well known that a lowered body temperature (hypothermia), can protect the brain against hypoxic and ischemic damage. The protective effect has been assumed to be due to a decreased brain metabolism, proportional to the decrease in temperature.

Brain oxygen consumption in hypothermia. The oxygen consumption in hypothermia has been studied both in vitro (Field et al. 1944) and in vivo (Rosomoff and Holaday 1954, Bering 1961, Michenfelder and Theye 1968). There are also a limited number of clinical observations (Stone et al. 1956, Cohen et al. 1964). All the studies agree in showing a decreased brain metabolism at lowered body temperature.

The quantitative relationship between brain metabolism and temperature is usually expressed by the term Q_{10} . Q_{10} is the quotient between the cerebral metabolic rate for oxygen, CMR_{O_2} , at one temperature and the CMR_{O_2} at a $10^{\circ}C$ lower temperature. The figure for Q_{10} varies in the literature from 2.2 (Michenfelder

^x Hypoxia is defined as an insufficient oxygenation of the tissues caused by decreased oxygen tension or oxygen content of the arterial blood, while ischemia is defined as a "critical" decrease in tissue perfusion.

and Theye 1968) to 4.6 (Cohen et al. 1964). Q_{10} will be constant over the whole range of temperatures only if there is a logarithmic relationship between CMR_{O_2} and temperature. Field et al. (1944) and Michenfelder and Theye (1968) found a linear relationship between $\log CMR_{O_2}$ and temperature $^{\circ}C$, while Bering (1961) found a linear relationship between the logarithm of CMR_{O_2} and $1/T$ absolute. Rosomoff and Holaday (1954) obtained a straight line when they plotted the numerical value for CMR_{O_2} against temperature ($^{\circ}C$).

All studies thus agree in showing that CMR_{O_2} decreases with temperature, but there are quantitative differences in Q_{10} between the different studies.

Brain oxygen consumption during hyperthermia. Field et al. (1944) found that the metabolism in brain tissue *in vitro* increased at temperatures above $37^{\circ}C$. Heyman et al. (1950) found no increase in CMR_{O_2} in patients whose body temperature was increased by $2^{\circ}C$ during fever therapy for asymptomatic neurosyphilis. Nemoto and Frankel (1970) found no increased CMR_{O_2} when body temperature was raised by $2.4^{\circ}C$ in dogs. However, when the temperature was raised by $4.4^{\circ}C$, CMR_{O_2} increased significantly (by 26 %). Demers et al. (1969) found an even larger increase during similar experiments. On the other hand, Hales (1973) claims that he found no increase in CMR_{O_2} in a study in unanaesthetized sheep, which were exposed to a high environmental temperature and thereby increased their body temperature.

It is more difficult to study hyperthermia because there seems to be a limit between 42 and $43^{\circ}C$, where brain metabolism again decreases. The temperature range which it is possible to study thus becomes more restricted. Field et al. (1944) found that brain tissue metabolism *in vitro* decreases with time at temperatures above $40^{\circ}C$. Nemoto and Frankel (1970) reported that CMR_{O_2} decreased when body temperature was increased from 42.1 to $43^{\circ}C$. Shapiro et al. (1973) showed that, in dogs, a body temperature above $43^{\circ}C$ led to a mortality greater than 50 %.

Intermediary metabolism. Measurements of oxygen uptake give no information about whether the oxygen supply to the tissue is adequate or not. It is therefore of interest to study the results from measurements of intermediary metabolism. As the organic phosphates (phosphocreatine, ATP, ADP and AMP) give information about whether or not the production and consumption of energy is in balance, they are of special interest. Such studies, as well as studies of glycolytic and citric acid cycle metabolites, are also of interest as they might reveal which mechanisms regulate the energy production in relation to the energy demand. However, there are only a few studies of intermediary metabolism in hypothermia or hyperthermia. During hypothermia unchanged concentrations of ATP, ADP and AMP, as well as an increase in phosphocreatine in the brain tissue have been reported (Thorn et al. 1958, Lowry et al. 1964, Sarajas et al. 1968, Brunner et al. 1971). This would indicate that there is a balance between production and utilization of energy in hypothermia, but the results are based on rather imprecise measurements. On the other hand, Goldberg et al. (1966) found decreases in phosphocreatine and ATP, and increases in ADP and AMP during hyperthermia, which would mean that consumption is greater than production of energy.

Glycolytic and citric acid cycle metabolites have been incompletely studied in both hypo- and hyperthermia. Lowry et al. (1964), in a study of mice cooled to 18°C, found increased concentrations in glucose-6-phosphate (G-6-P) and fructose-6-phosphate (F-6-P) and a decrease in fructose diphosphate (FDP). This finding would indicate inhibition at the phosphofructokinase step (see Discussion). Thorn et al. (1958) also found decreases in FDP during hypothermia. Goldberg et al. (1966), in a study of hyperthermia, found a pronounced increase in pyruvate, and moderate increases in α -ketoglutarate (α -KG), fumarate and malate. However, except for the results reported by Lowry et al. (1964) in hypothermia, none of the referred studies gives conclusive information about the mechanisms which regulate the metabolism in

relation to the energy demand during hypo- or hyperthermia.

Ventilation during hypothermia. Cerebral blood flow (CBF) changes with alterations in PCO_2 . The quantitative relationship between CBF and PCO_2 is well studied during normothermia (Kety and Schmidt 1948b, Wasserman and Patterson 1961, Novack *et al.* 1953, Reivich 1964, Harper and Glass 1965). During hypothermia, however, there are only a few incomplete reports (Hornbein *et al.* 1969, Forrester *et al.* 1964), indicating that the decrease in CBF during hypocapnia is of the same magnitude in hypothermia as in normothermia. The PCO_2 response of CBF is of importance in the question of ventilatory management during hypothermia. Two ventilatory patterns, leading to different levels of PCO_2 , are usually discussed in the literature. 1. Regulation of the ventilation so that pH in arterial blood remains constant. This is achieved by adding CO_2 to the inspired gases or by decreasing the ventilatory volume during hypothermia. This pattern leads to a slight decrease in PCO_2 . 2. The second possibility is to regulate the ventilation so that PCO_2 is normal in normothermia and let this ventilatory volume remain constant also during hypothermia. As metabolism decreases in hypothermia, CO_2 production decreases, and, therefore this pattern of ventilation leads to a marked decrease in PCO_2 . However, as the solubility of CO_2 increases with decreasing temperature, the CO_2 content in the blood remains relatively constant. Severinghaus (1959) suggested that the constant CO_2 content reflects balance between production and elimination of CO_2 and therefore advocated that the ventilatory volume should be unchanged when hypothermia is induced. However, if the PCO_2 response of CBF is preserved during hypothermia, this method would cause a marked reduction in CBF.

While hypothermia is not a physiological condition for man, some animals regularly change their temperature. The hibernators have a body temperature of $37^{\circ}C$ in their active periods. During hibernation, however, the temperature decreases to about $5^{\circ}C$. Kent and Peirce (1967) studied blood gases and acid-base characteristics

in ground-squirrels at a body temperature of 37°C and 5°C . They found that the animals regulated their ventilation during hibernation, so arterial pH remained constant, P_{CO_2} decreased slightly (from 42 to 35 mm Hg) and total CO_2 content in the blood increased by 50 %. Reeves (1969) confirmed these findings in a study in hibernating bats, hamsters, sloths, and ground-squirrels.

There are also clinical reports showing that spontaneously ventilating patients during hypothermia regulate ventilation so that pH remains constant and P_{CO_2} is slightly reduced (Lougheed et al. 1955).

Objectives of the present study. The above discussion shows that important questions remain to be answered about the influence of hypothermia and hyperthermia on brain energy metabolism and blood flow.

1. What is the quantitative relationship between changes in body temperature and CMR_{O_2} ? What is the value for Q_{10} ?

2. How will changes in body temperature influence intermediary metabolism? Is there a balance between production and utilization of energy? What mechanisms regulate the changes in metabolism in hypothermia and hyperthermia?

3. How should the ventilation be regulated during hypothermia? Is the P_{CO_2} response of CBF unchanged during hypothermia, and if so, could it be disadvantageous to hyperventilate patients during hypothermia?

METHODS

All experiments were performed on male Wistar rats (Møllegaard Hansens Avlslaboratorier, Denmark). The rats were fed and had free access to tap water until operation. During the experiments they were ventilated with 30 % oxygen and 70 % N_2O . In hypothermia, ventilation was regulated so that pH remained constant.

Measurements of cerebral blood flow. CBF was measured with the Kety and Schmidt (1948a) technique, modified for use in rats by Eklöf et al. (1973) and Norberg and Siesjö (1974). The principles can be summarized as follows. $^{133}\text{Xenon}$ is added to the inspired gases ($\text{O}_2/\text{N}_2\text{O}$, 30/70), which are supplied to the animal from a rubber bag, attached to the respirator. After 15 minutes of Xenon supply (the saturation phase), the brain is saturated with $^{133}\text{Xenon}$, i.e. there is equal activity in blood and tissue. Samples are taken from the femoral artery and the superior sagittal sinus. The samples are analyzed for arteriovenous difference in $^{133}\text{Xenon}$ and oxygen content. The supply of Xenon is then interrupted, and the animal is ventilated only with oxygen and nitrous oxide (desaturation phase). Repeated samples are taken from the femoral artery and the superior sagittal sinus. The activity of $^{133}\text{Xenon}$ is measured. The values are calculated as per cent of the value obtained at the end of the saturation period, and plotted against time (see Fig. 1)

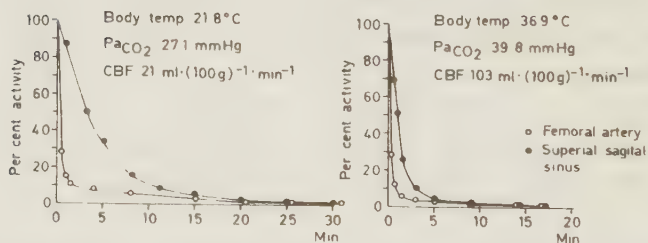


Fig. 1. Representative desaturation curves for $^{133}\text{Xenon}$ in hypothermia (22°C) and normothermia (37°C).

Fig. 1 shows that the activity falls rapidly in arterial blood and slower in venous blood. The arterial and venous curves come together after 5-10 minutes in normothermia and 15-20 minutes in hypothermia. CBF is calculated according to the formula

$$CBF = \frac{C_{v0} \cdot \lambda \cdot 100}{\int_0^T (C_a - C_v) dt}$$

C_{v0} is the activity of venous blood immediately before desaturation, λ is the partition coefficient for $^{133}\text{Xenon}$ between tissue and blood, and the expression $\int_0^T (C_a - C_v) dt$ is the integral of the arterio-venous differences in $^{133}\text{Xenon}$ activity, i.e. the area between the arterial and venous curves.

The Kety and Schmidt technique is based on the Fick principle, that is, on the law of conservation of matter, and therefore gives a quantitative measure of CBF provided that certain requirements are fulfilled. 1. CBF must be constant during the measurements. 2. There must be equilibrium in $^{133}\text{Xenon}$ activity between blood and tissue both at the end of the saturation and the desaturation period. 3. There must be no extracerebral contamination in the venous blood. 4. The partition coefficient, λ , for $^{133}\text{Xenon}$ between blood and tissue must be known.

Condition 1. is controlled by making two determinations of arterio-venous differences in oxygen content ($a - v D_{O_2}$), one immediately before desaturation and one in the middle of the desaturation period. The values for $a - v D_{O_2}$ must not differ by more than 10 %. Condition 2. and 3. are fulfilled if there is no arterio-venous difference in $^{133}\text{Xenon}$ activity either at the end of the saturation or at the end of the desaturation period (see Norberg and Siesjö 1974).

The partition coefficient, λ , has previously been determined at 37°C by Veall and Mallett (1965). Changes of λ with temperature

cannot be excluded, and, in paper I, we therefore determined λ at 37, 32, 27 and 22°C. λ decreased with temperature from a value of 0.83 at 37°C to 0.68 at 27°C.

As the superior sagittal sinus mainly drains cortical tissue, the CBF method we have used will quantitatively measure blood flow and oxygen uptake in cortical (grey) tissue.

Measurements of total oxygen content in blood. Total oxygen content (T_{O_2}) in arterial and cerebral venous blood was measured on 25 μ l samples using the potassium ferricyanide method of Fabel and Lübberts (1964), based on a commercial P_{O_2} electrode. The samples were delivered directly from the sampling capillaries to the electrode chamber by attaching the capillaries to a Hamilton syringe. A solubility factor of 0.0326 ml/(mm Hg·100 ml) was used to convert P_{O_2} to T_{O_2} . The method quantitatively measures the total oxygen content and gives a reproducibility, which is usually better than 2 per cent (Borgström et al. 1974).

Calculation of CMR_{O_2} . T_{O_2} is measured in arterial and cerebral venous blood in the middle of the desaturation phase. a-v D_{O_2} is calculated, and CMR_{O_2} is determined according to the formula

$$CMR_{O_2} = CBF \cdot AVD_{O_2}$$

From the formula it follows that if CMR_{O_2} is constant, changes in CBF can be determined.

$$CBF = \frac{CMR_{O_2}}{a-v D_{O_2}} = \frac{\text{constant}}{a-v D_{O_2}}$$

Freezing technique and metabolite analysis. The metabolism of the brain is high and the tissue contains only a small amount of oxygen. When the oxygen supply to the brain is interrupted, autolytic changes will therefore occur very rapidly. In metabolic studies of brain tissue, methods therefore have to be used which minimize

these autolytic changes. In previous metabolic studies in hypo- and hyperthermia, methods have been used where unanaesthetized animals have been thrown into liquid nitrogen (Lowry et al. 1964, Goldberg et al. 1966, Sarajas et al. 1968, Brunner et al. 1971). This method, however, gives autolytic changes even when used in small animals like mice (Pontén et al. 1973 a, b).

In order to minimize artefacts, we have used the freezing-funnel technique (see Pontén 1966, Granholm and Siesjö 1971, Pontén et al. 1973a). The animals were artificially ventilated. Blood pressure and body temperature were continuously followed. Arterial P_{O_2} , P_{CO_2} and pH were measured intermittently. Towards the end of the experiment, a skin incision was made on the skull. A plastic funnel was fitted into the skin incision, directly over the skull bone, and the brain tissue was frozen by pouring liquid nitrogen into the funnel. The animals were ventilated during the freezing period, and the blood pressure did not fall until after about 2 minutes. Pontén et al. (1973) have also shown that the circulation is maintained almost to the moment when the tissue freezes.

The brains were chiselled out at the temperature of liquid nitrogen, and the tissue was extracted at temperatures below 0°C (see Folbergrová et al. 1972a). Enzymatic fluorometric micro-methods were used for analysis of glycolytic and citric acid cycle metabolites, as well as for organic phosphates and amino acids (for details, see Lowry and Passonneau 1972, Folbergrová et al. 1972 a, b, 1974 a, b).

RESULTS AND DISCUSSION

I The effect of induced hypothermia upon oxygen consumption in the rat brain.

Four groups of experimental animals were studied. Three of the groups were cooled to 32 , 27 and 22°C by surface cooling, while the fourth group was maintained at 37°C . The animals were ventilated so

that pH was kept constant. After 45 minutes at the experimental temperature, CBF and a-v D_{O_2} was measured and CMR_{O_2} calculated. CMR_{O_2} decreased linearly with temperature between 37 and 22°C (Fig. 2).

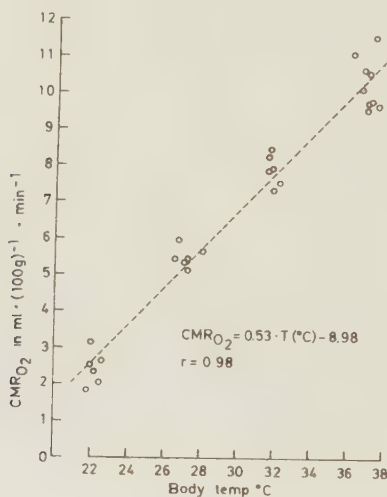


Fig. 2. The temperature dependence of CMR_{O_2} . The regression line is drawn, and the equation for the line is given in the figure.

CBF decreased in parallel with the decrease in CMR_{O_2} , but there was a larger scatter of values, especially at 32 and 27°C (Fig. 3). Our results are in agreement with those reported by Rosomoff and Holaday (1954). These authors also found a linear relationship between CMR_{O_2} and temperature, and an almost parallel decrease in CBF.

Bering (1961) and Michenfelder and Theye (1968) found a logarithmic relationship between CMR_{O_2} and temperature. These authors claimed that their results are in accordance with an Arrhenius

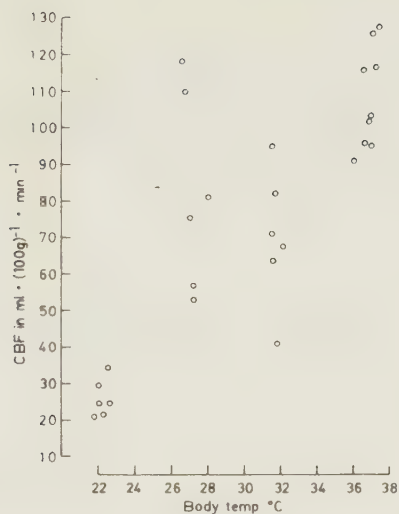


Fig. 3. Individual CBF values in the temperature range 37-22°C.

plot or a van't Hoff isocore. However, during scrutiny of Bering's data, in which the values are rather scattered, it is just as possible to fit the points within the range 37-25°C into a straight line. Michenfelder and Theye (1968) reported only a few values over a limited temperature interval, so that it is difficult to draw conclusions from their data.

The velocity of a single enzyme reaction in vitro follows an Arrhenius plot, i.e. the logarithm for the velocity, when plotted against $\frac{1}{T \text{ absolute}}$, gives a straight line. Bering (1961) is of the opinion that this is also the relationship that one would expect between $\dot{C}MR_{O_2}$ and temperature. He then assumes that the decrease in $\dot{C}MR_{O_2}$ is due to the temperature effect on one single regulating enzyme. It is more reasonable to assume that the decreased temperature leads to reduced demand on energy production in the tissues, and that energy requirements determine the metabolic rate.

A simple relationship between CMR_{O_2} and temperature is therefore not to be expected.

II Effect of hypothermia upon organic phosphates, glycolytic metabolites, citric acid cycle intermediates and associated amino acids in rat cerebral cortex.

Two series of experiments, series A and series B, were performed. In series A there were four groups. Three of them were cooled to 32, 27 and 22°C, while the fourth was maintained at 37°C. After one hour at the experimental temperature, the brain was frozen. Simultaneously, arterial blood and CSF samples were also taken, and frozen in liquid nitrogen. Series B consisted of three groups, one at normothermia and two groups cooled to 22°C. However, these animals in series B were maintained for two hours, instead of one hour, at the experimental temperature before the brains were frozen. In one of the hypothermic groups in series B, nitrous oxide was exchanged for nitrogen and these animals were ventilated with O_2/N_2 , 30/70 instead of $\text{O}_2/\text{N}_2\text{O}$, 30/70.

We found no influence of hypothermia on the tissue concentrations of the organic phosphates, except for a moderate increase in phosphocreatine (PCr). This finding is in agreement with other studies (Thorn et al. 1958, Lowry et al. 1964, Brunner et al. 1971). However, we found a smaller increase in PCr than the others have reported, which is probably due to the fact that our freezing technique gives less autolytic changes. As the concentrations of ATP, ADP and AMP were unchanged, it is doubtful if the increases in PCr were due to a changed balance between production and utilization of energy. Previous studies have shown that the creatinphosphokinase equilibrium, and thus also PCr concentration, is pH dependent (Siesjö et al. 1972). It is thus possible that an intracellular alkalosis causes the increases in PCr concentration. This theory is supported by the findings in a recent study by Nilsson et al. (1975), who reported unchanged concentrations of all the organic

phosphates including PCr at 22°C. They also used the freezing-funnel technique but had a constant P_{CO_2} in arterial blood.

Fig. 4 shows the changes in glycogen, glucose and the glycolytic and citric acid cycle intermediates at 32, 27 and 22°C. The values are given as per cent changes from the control values at 37°C.

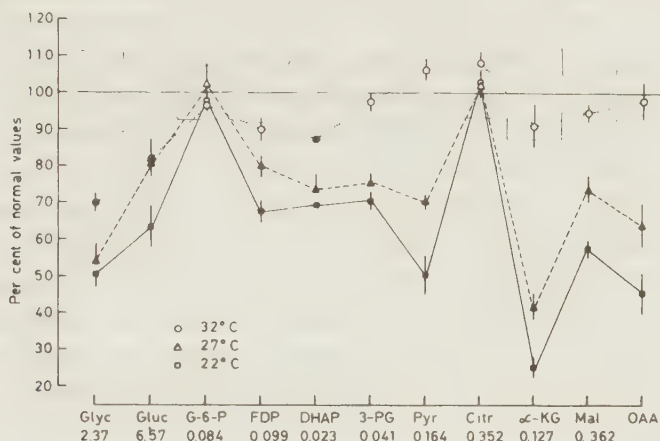


Fig. 4. Glycolytic and tricarboxylic acid cycle substrate changes after 1 hour at 32°C, 27°C and 22°C. Each point gives the per cent change from the control value as mean $\pm$ S.E.M. The mean control values are given at the bottom of the figure as $\text{mmols} \cdot \text{kg}^{-1}$ wet tissue for all the substrates except glucose and pyruvate, which are given as derived intracellular concentrations. The thin horizontal lines indicate 95 per cent confidence limits for the control groups. Filled symbols indicate significant difference from control values ($p < 0.05$) as tested with student's t-test. Six animals in each group.

At all the temperatures studied, there was a decrease in glycogen and glucose concentrations in the brain tissue. At 32°C, there were no other significant changes, but with further decreases in temperature there was a fall in all the metabolites, except for glucose-6-phosphate (G-6-P) and citrate. The reduction was most pronounced in glycogen and α -ketoglutarate (α -KG). The results from series B showed a slightly more pronounced pattern. On the other hand, there were no differences between the animals ventilated with O_2/N_2O and the unanaesthetized animals ventilated with O_2/N_2 .

The lowered concentrations of glycogen and glucose, and the unchanged concentrations of organic phosphates (except for the increased PCr) shows that hypothermia is not associated with an increased supply of energy (actual or potential).

The lowered concentrations of glycogen in hypothermia have been assumed to be due to cold stress (Hutchins and Rogers 1970). Unpublished data from our laboratory show, however, that glycogen decreases in hypothermia, even in adrenalectomized rats. It therefore seems possible that the decreased level of glycogen depends on a temperature effect on the enzymes catalyzing the synthesis of glycogen.

Glucose is stepwise metabolised in the glycolytic chain and citric acid cycle in reactions catalyzed by enzymes. Normally, there is a surplus of enzymes. The flux of substrate seems to be determined by certain so called "regulatory" enzymes. The activity of the regulatory enzymes varies with the energy demand. The reactions they catalyze are not in thermodynamic equilibrium, and they are influenced by factors other than the concentration of their substrate. Glycolytic enzymes belonging to this regulating group are hexokinase, phosphofructokinase and pyruvate kinase. In the citric acid cycle, very little is known about enzymatic control of the metabolism, but isocitrate dehydrogenase is supposed to be one of the regulatory enzymes (see Newsholme and Start 1973). Phosphofructokinase, which is thought to be the main regulator of the glycolytic substrate flux, is activated by, for instance, ADP, AMP and inorganic phosphate, while it is inhibited by ATP and

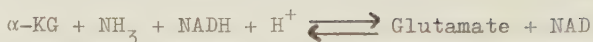
citrate. If an energy-requiring process is taking place, ATP is consumed and ADP and AMP are formed. This would activate phosphofructokinase so that the glycolytic flux is increased and more ATP is formed. A decreased ATP consumption on the other hand, would lead to a decreased glycolytic flux.

Krebs (1957) postulated that a regulatory enzyme can be identified during non-steady state conditions, as the substrate of the enzyme will increase in concentration when the substrate flux decreases, and inversely, when the substrate flux increases, the substrate of the regulatory enzyme will decrease. If, for instance, the glycolytic flux decreases because of an inhibition of phosphofructokinase, one would expect an increase in its substrate, fructose-6-phosphate (or of glucose-6-phosphate, which is in equilibrium with F-6-P).

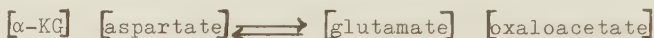
In our study we found a decrease of 20-50 % in concentrations of the glycolytic and citric acid cycle metabolites. At this new level, we have relative, although not absolute, increases in F-6-P and citrate. Citrate and isocitrate are supposed to be in equilibrium. If this is true also in hypothermia, we can conclude that phosphofructokinase and isocitrate dehydrogenase are regulatory enzymes that retard substrate flux to match the (lowered) requirements for energy.

The metabolites of the citric acid cycle are closely related to some amino acids. We found decreased concentrations of glutamate and increased concentrations of aspartate and alanine. The concentrations of γ -aminobutyric acid (GABA) and glutamine were unchanged. Our results are in agreement with those reported by Turinsky et al. (1971) in hypothermic rats. On the other hand, they disagree with values reported by Mandel et al. (1966), who found decreases in aspartate and glutamate, and increases in GABA and glutamine in hibernating garden door mice. However, while we found a markedly increased concentration of ammonia in the hypothermic rat, this has not been reported in hibernating animals (Godin et al. 1967). The first step in detoxifying ammonia in the brain tissue is the

amination of α -KG to glutamate



A high concentration of ammonia shifts the reaction to the right and this causes a decrease in α -KG, which is also in agreement with our findings. The lowered α -KG will cause a shift in the aspartate aminotransferase equilibrium



This reaction will be shifted to the left and the concentration of glutamate will decrease and aspartate will increase.

In order to replace the loss of α -KG, we will also get a shift in the alanine aminotransferase reaction



to the left and the concentration of alanine will increase, while glutamate decreases.

The high concentrations of ammonia in the hypothermic animals remain to be explained.

III Influence of changes in arterial PCO_2 on cerebral blood flow and cerebral energy state during hypothermia in the rat.

In this paper, the relation between PCO_2 and CBF was studied during hypothermia. The body temperature was decreased to 22°C in artificially ventilated rats and CBF was determined from the arterio-venous difference in oxygen content at PCO_2 values of 15, 30, 40 and 60 mm Hg, respectively. The results were compared to those of a normothermic group. When oxygen uptake is constant, changes in $a\text{-v } \text{D}_{\text{O}_2}$ give good information about changes in CBF (see Introduction). Eklöf *et al.* (1973) have shown that CMR_{O_2} does not change with changes in PCO_2 in normothermia. In order to see if this is also true in hypothermia, we determined CBF and CMR_{O_2} at 22°C and PCO_2 13.8 mm Hg. The value for CMR_{O_2} did not significantly differ from a previously determined value at 22°C and PCO_2 25.9 mm Hg. We thus conclude that our method, of following CBF by changes in

a-v H_2O_2 , was valid.

Fig. 5 shows that when P_{aCO_2} is increased from 40 to 60 mm Hg, we get a parallel increase in CBF during hypothermia and normothermia. When P_{aCO_2} was decreased from 40 to 15 mm Hg, however, we get a much more pronounced reduction in CBF during hypothermia.

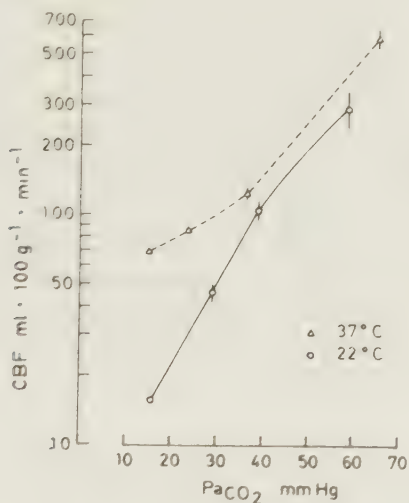


Fig. 5. Changes in CBF with P_{aCO_2} in normothermia and hypothermia. CBF is plotted on a logarithmic scale. S.E. is given when larger than the symbol. Six animals in each group.

Our results in normothermia are in agreement with those of previous studies. The fall in CBF during hypocapnia is reduced at P_{aCO_2} values lower than 20 mm Hg (Selvick 1964, Wollman et al. 1968). During normothermia we found only a small decrease in CBF when P_{aCO_2} was reduced from 22.5 mm Hg to 15 mm Hg. It has been assumed that the reduction in CBF is so pronounced at P_{aCO_2} values lower than 20 mm Hg that the tissue will become hypoxic, and that this hypoxia would counteract further vasoconstriction. This theory is supported by the findings of MacVillan and *et al.* (1973), who found a small

reduction in PCr and a small, but significant, increase in ADP at P_{CO_2} 10 mm Hg.

In hypothermia we got a much more pronounced reduction in CBF when P_{CO_2} was lowered from 30 to 15 mm Hg. At P_{CO_2} 15 mm Hg and a body temperature of $22^{\circ}C$, we found a CBF value which was less than 20 % of the value found in normothermia and normocapnia. It should be recalled that if we lower the temperature in a normocapnic animal from $37^{\circ}C$ to $22^{\circ}C$, without changing the ventilatory volume, we will get a P_{CO_2} of about 15 mm Hg. In order to see if the very low CBF obtained at that P_{CO_2} could provide the tissue with sufficient oxygen, we determined organic phosphates and lactate in brain tissue from animals at $22^{\circ}C$ and P_{CO_2} 15 mm Hg. The results were compared to those of another hypothermic group, which had a P_{CO_2} value of 30 mm Hg. We found no differences in PCr, ATP, ADP, AMP or the ATP/ADP ratio between the two groups. In the hypocapnic group the lactate concentration increased from 0.94 to 1.62 mmol/kg. This increase is much smaller than the corresponding increase in normothermia.

Although we have not been able to demonstrate any harmful effects of hypocapnia during hypothermia in the normal rat, it is not certain that this applies in pathological conditions. In our opinion, it is safer to avoid extreme hypocapnia in patients with central nervous system disease or damage.

We have regulated the ventilation in hypothermia so that pH has remained constant. The following reasons are in favour of this method: 1. Hibernating animals, as well as spontaneously ventilating man, regulate their ventilation so that pH remains constant in hypothermia. 2. If we regulate the ventilation in this way, we get a parallel decrease in CBF and CMR_{O_2} with decreases in temperature. 3. Extreme hyperventilation in hypothermia leads to a very pronounced reduction in CBF.

1V. The effect of hyperthermia upon oxygen consumption and upon organic phosphates, glycolytic metabolites, citric acid cycle intermediates and associated amino acids in the rat cerebral cortex.

Two series of experiments were performed. In the first series CBF and CMR_{O_2} were determined after 30 minutes at 40 and 42°C. In the second series, the brains were frozen for tissue analysis after 30 minutes at the same temperatures. One group of animals was frozen as soon as the temperature had reached 42°C. In both series, a control group was maintained at 37°C. The animals were heated by a lamp bulb under the operation table. As all the animals at 42°C had a metabolic acidosis, it was not possible to regulate ventilation so that pH remained within the normal range, and therefore arterial CO_2 tension was maintained constant instead.

Fig. 6 shows the changes in CMR_{O_2} with increased temperature.

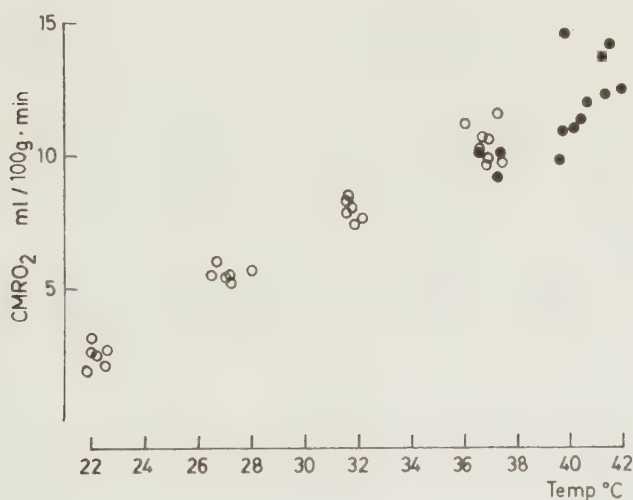


Fig. 6. Individual CMR_{O_2} values in the temperature range 22-42°C. Filled circles are from the present study and open circles from paper I.

For comparison, the data from the hypothermic study are also given in the figure. When the temperature was raised, CMR_{O_2} increased by 5-6 % per degree C. This is in good agreement with the value found for decreasing temperature. In hyperthermia there was also an almost parallel increase in CBF.

The results from the second series showed that there were no changes in organic phosphates that would have given evidence of an imbalance between consumption and production of energy. Goldberg *et al.* (1966) found marked changes in the organic phosphates in hyperthermia, but these changes are probably at least partly due to freezing artefacts (see Methods). Nilsson *et al.* (1975), inducing an increase in temperature to 42°C , reported changes in ATP, ADP and AMP (non-significant) and in PCr (significant) that were suggestive of a disturbed balance between production and consumption of energy. In their material, there was also a rise in lactate/pyruvate ratio. It seems plausible that 42°C represents a critical temperature above which a derangement of energy metabolism occurs (see Introduction).

After 30 minutes at the experimental temperature, there was an increase in glucose and G-6-P in the hyperthermic groups. At 42°C there was also an increase in F-6-P. Glycogen did not change, neither did the other glycolytic or citric acid cycle metabolites. The only change in amino acids was an increase in glutamate concentration.

In order to see if there were any metabolic changes with time one group was frozen immediately when the temperature reached 42°C . However, we found only small differences between this group and the one frozen after 30 minutes.

The results cannot be readily explained in terms of activation of regulatory enzymes. Thus, since an increased CMR_{O_2} would suggest that glycolytic flux also is increased, activation of PFK should have manifested itself in decreases in F-6-P and G-6-P (Krebs theorem). The results are similar to those obtained in hypoxia, during which there are signs of initial activation of PFK, later

to be followed by increases in G-6-P and F-6-P (Norberg and Siesjö 1975). Probably in hypothermia (which is induced gradually) the increased levels of G-6-P and F-6-P indicate that hexokinase has been activated to a larger extent than PFK.

This explanation is tentative and it has not been conclusively shown that hyperthermia is associated with an increase in glucose consumption. Thus, under special circumstances, the brain can use substrates other than glucose. During starvation for instance the brain can utilize β -hydroxybutyrate and acetoacetate, since there are enzymes that can metabolise these keto acids to acetyl CoA (see Ruderman et al. 1974, Ferrendelli 1974). Normally there are very low levels of keto acids in the blood, but if such acids are formed in hyperthermia, they, as well as glucose, could be used as substrate by the brain. In that case, glucose metabolism could be unchanged or even decreased. We found an increased brain to blood glucose ratio at 42°C. This could be due to an increased transport of glucose into the brain because of temperature influence on the carrier mechanism for glucose, but it could also be due to a decreased utilization of glucose. The high glucose concentration could then secondarily activate hexokinase, which would explain the increased concentrations of G-6-P and F-6-P.

From the present study, it is thus not possible to conclude whether or not glucose metabolism increases in hyperthermia. Direct measurements of glucose metabolism would be desirable.

CONCLUSIONS

On the basis of the results discussed, the following conclusions can be drawn.

1. When temperature decreases from 37° to 22°C, CMR_{O_2} decreases linearly with temperature. As there is no logarithmic relationship between CMR_{O_2} and temperature, the term Q_{10} becomes less useful. We suggest that changes in CMR_{O_2} with temperature are best expressed as per cent change per degree C. In the temperature

range 37-22°C we found a 5 % decrease in CMR_{O_2} per degree C.

When the temperature was elevated from 37 to 42°C, there was a 5-6 % increase in CMR_{O_2} per degree C increase in temperature.

2. Since previous studies in hibernating and in homiothermic spontaneously breathing animals have shown that arterial plasma pH is maintained constant when temperature is decreased, an attempt was made to keep arterial pH at 7.4. However, in the hyperthermic animals P_{CO_2} was kept constant. Under these conditions, CBF varied in proportion to the changes in CMR_{O_2} .

With an increase in CO_2 tension in hypothermia, the response of CBF was similar to that observed in normothermia. With a decrease in CO_2 tension there was a much more pronounced decrease in CBF in hypothermia. At a body temperature of 22°C and an arterial CO_2 tension of 15 mm Hg, CBF was reduced to less than 20% of the normo-thermic, normocapnic value. Although this drastic fall in CBF was tolerated without metabolic signs of tissue hypoxia, it is concluded that excessive hyperventilation should be avoided in patients with brain diseases.

3. Even when methods of great sensitivity are used, there are no differences in tissue concentrations of ATP, ADP or AMP in hypo- or hyperthermia. Thus, there is a constant phosphorylation state over a wide temperature range.

There was no increase in glucose, and a fall in glycogen, and the wellknown protective effect of hypothermia in conditions of ischemia is therefore not related to increased carbohydrate or energy stores.

4. In hypothermia, there were increased levels of glucose-6-phosphate and citrate. These changes are probably caused by inhibition of phosphofructokinase and isocitrate dehydrogenase due to a decreased demand for energy.

In hyperthermia the pattern of changes in the glycolytic chain and the citric acid cycle does not permit conclusions about possible regulatory steps.

5. In hypothermia (22°C) there was a clear increase in tissue ammonia concentration. This increase may have accelerated the

amination of α -KG which can explain the low concentrations of α -KG. By shifting the alanine and aspartate aminotransferase reactions, the low concentration of α -KG is probably responsible for the increases in alanine and aspartate.

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